Gold Nanorods

in Biological Imaging and Sensing

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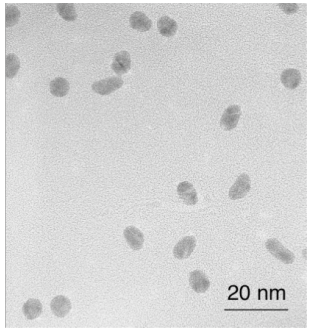
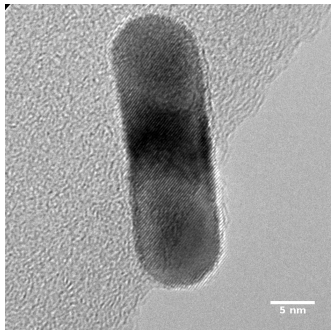
Content

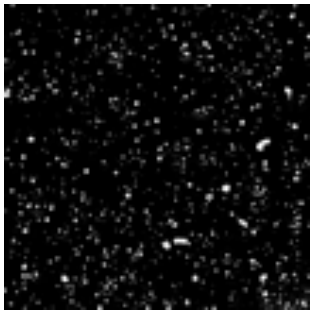
- Introduction
- Two-photon luminescence of gold nanorods in biological imaging
- Surface plasmon enhanced energy transfer in biomedical sensing

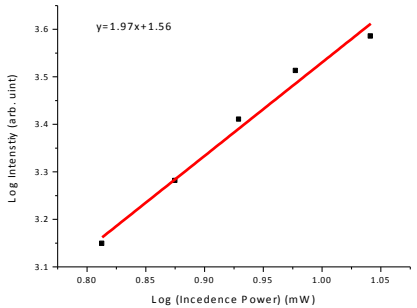
Synthesis



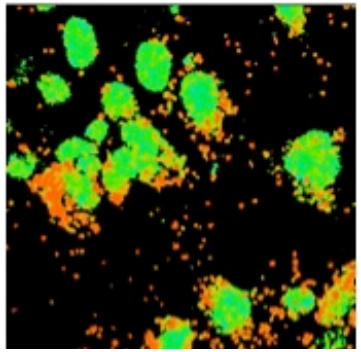


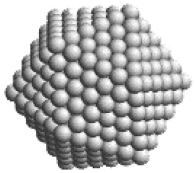
Luminescence





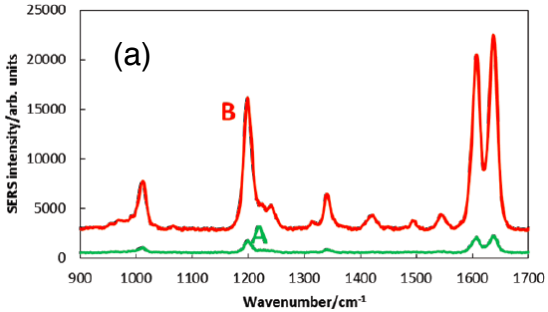
Bio-imaging



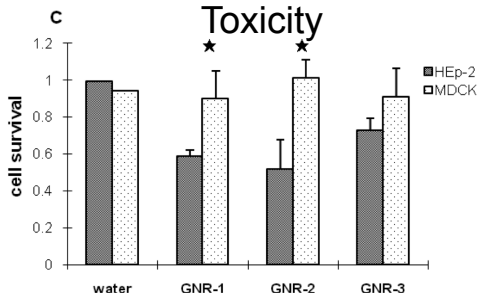


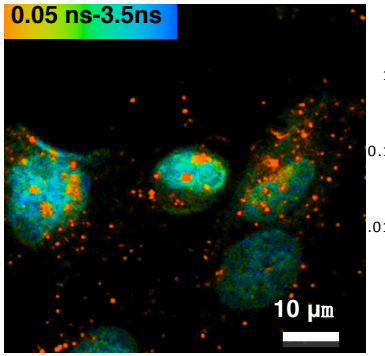
Noble Metal NP

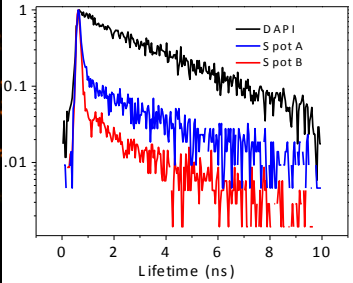
SERS

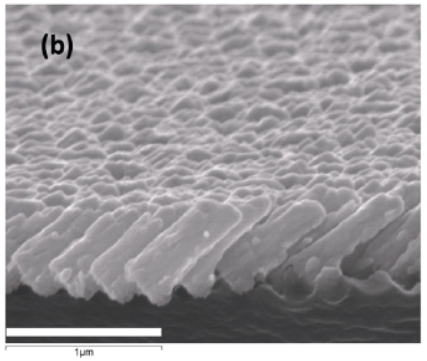


Bio-sensing









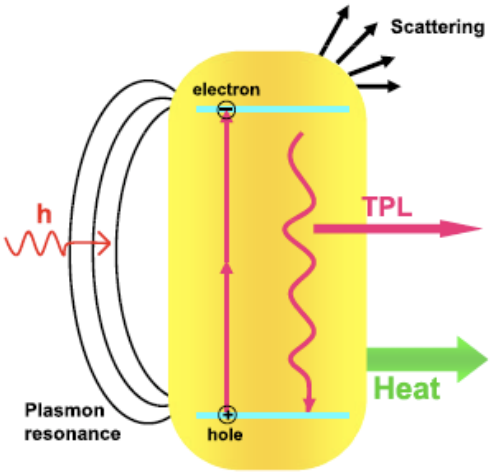
Biomedical Applications

Advantages:

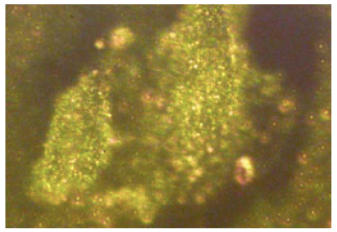
- Easily prepared
- Low toxicity
- Readily attached to biomolecules
- Unique optical properties
- No photobleach problems

Sensing

- Enhanced Raman Scattering
- Surface energy transfer

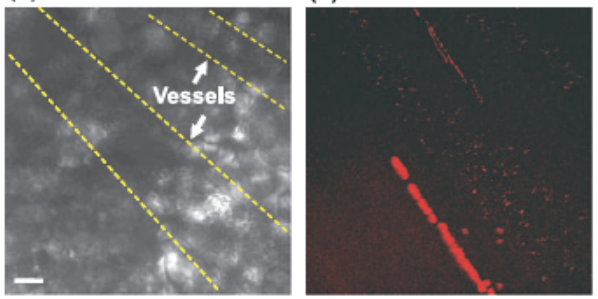


Imaging

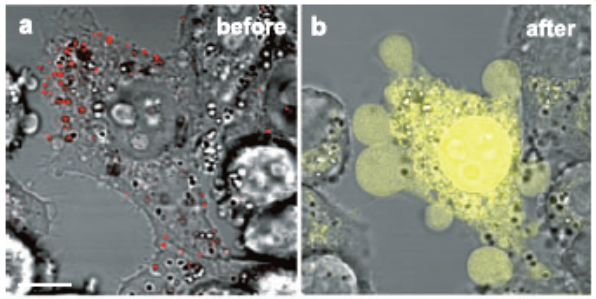


Contrast agent for cellular imaging by darkfield microscopy

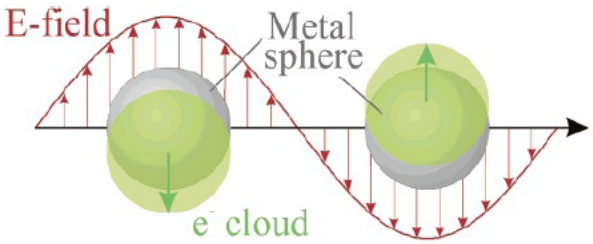
In – vivo two- photon imaging

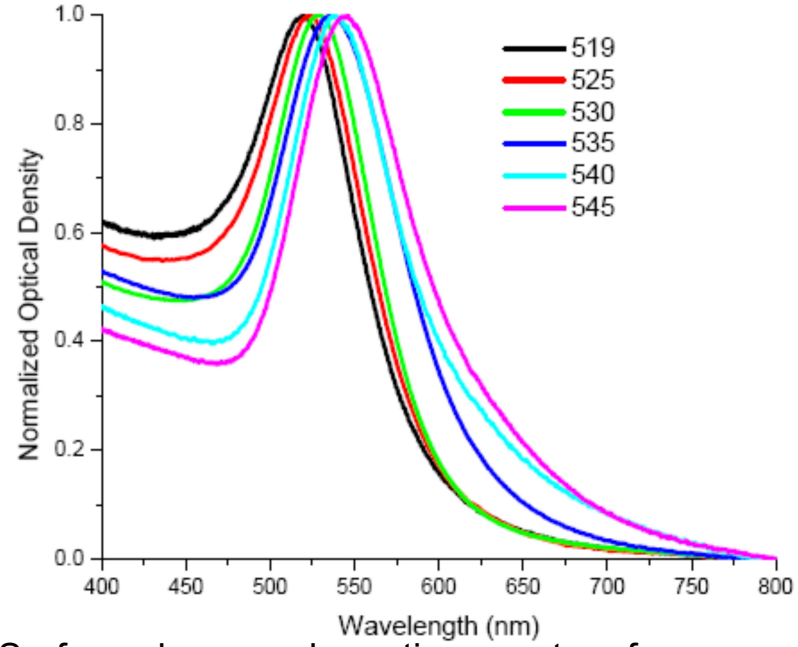


Photothermal therapy

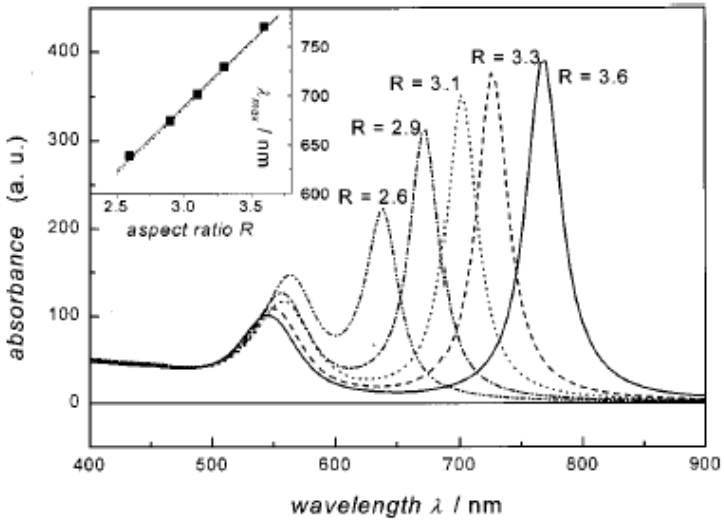


Surface Plasmon





• Surface plasmon absorption spectra of spherical gold nanoparticles in different sizes.

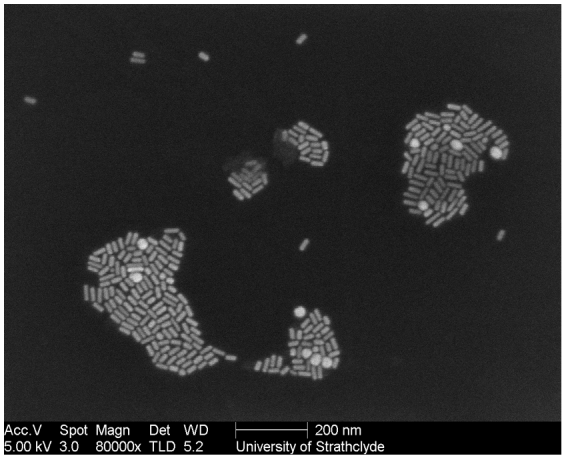


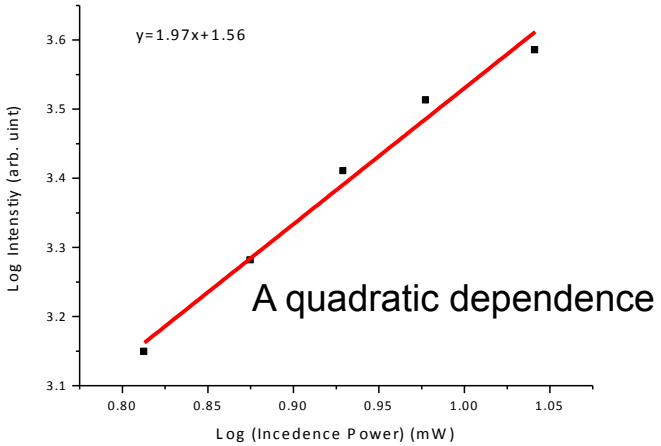
• Calculated absorption spectrum of elongated ellipsoids with varying aspect ratios R *[from S. Link, et al, J. Phys. Chem. B, 103, 3073 (1999)].*

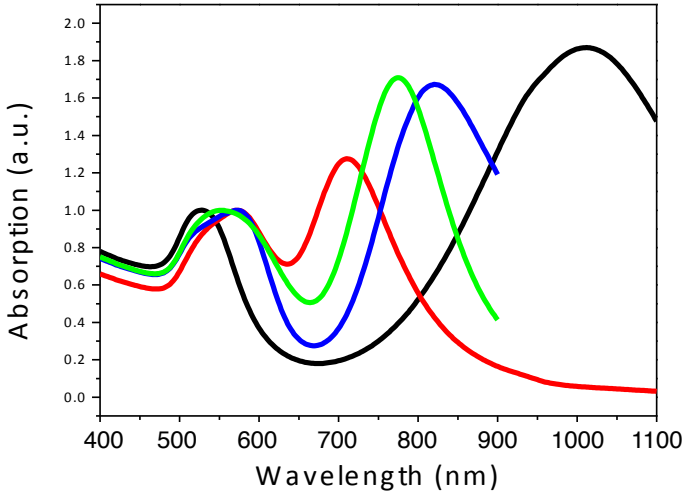
• Value of asymmetric nanoparticles (nanorods):

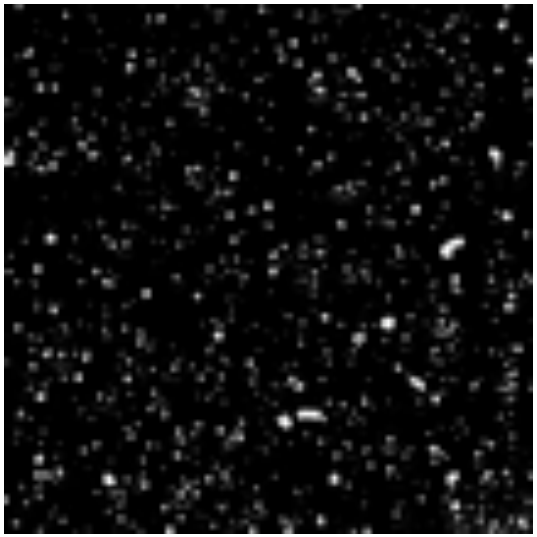
- Tunable wavelength
- Enhancement in the local electromagnetic field
- PL enhancement
- Polarization

Two Photon Luminescence (TPL) of Gold Nanorods







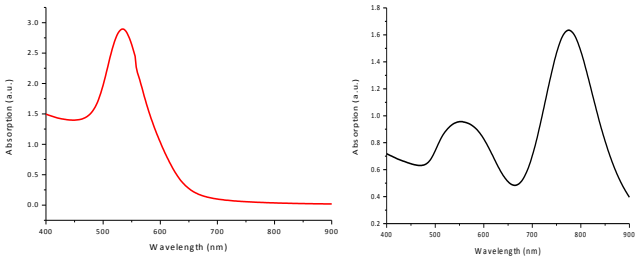


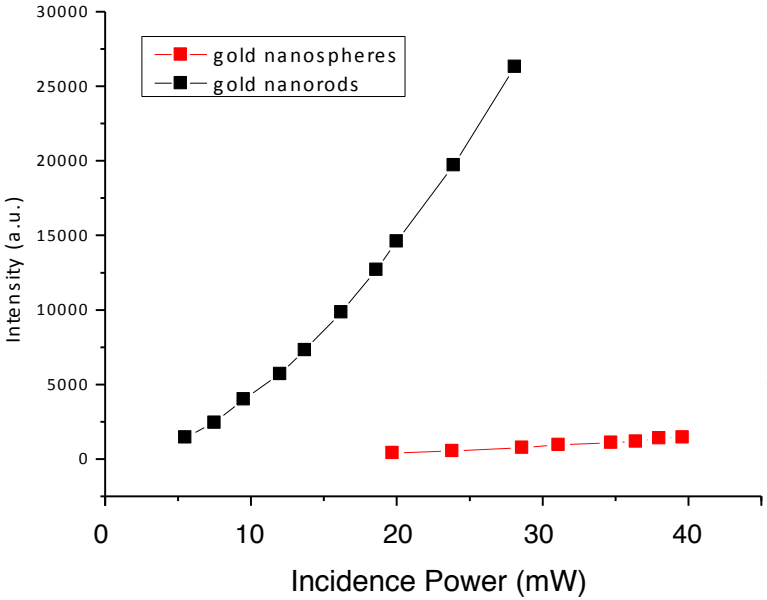
scanning area
133μm x 133μm

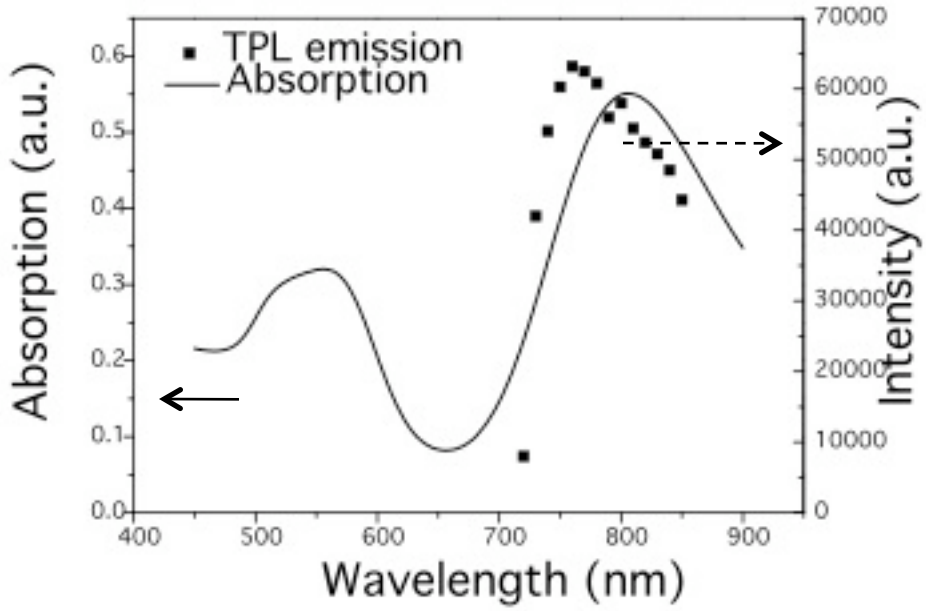
Ex 800nm, 80MHz

Bandpass filter
535 – 560nm

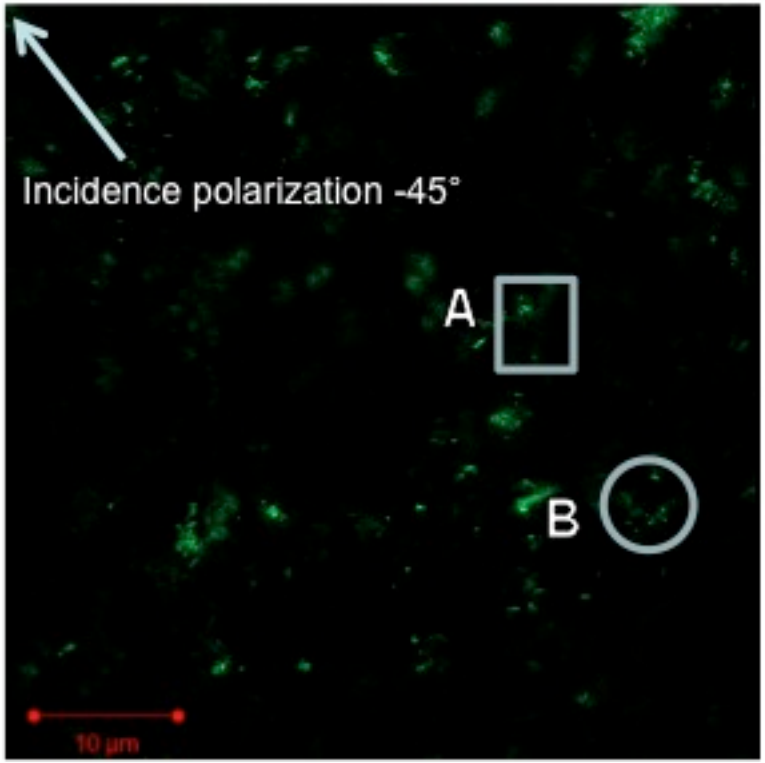
Two Photon Luminescence – shape

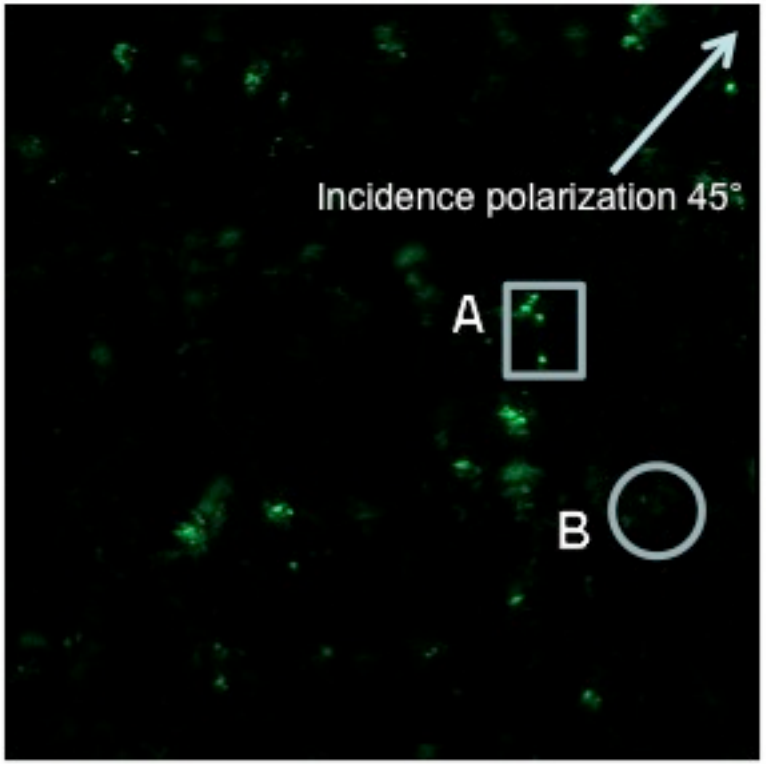






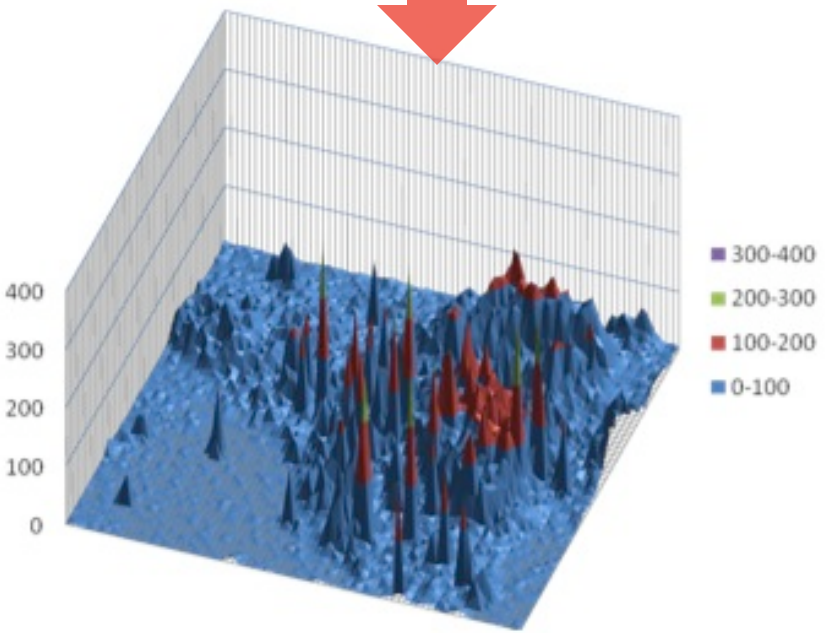
Two Photon Luminescence - polarization

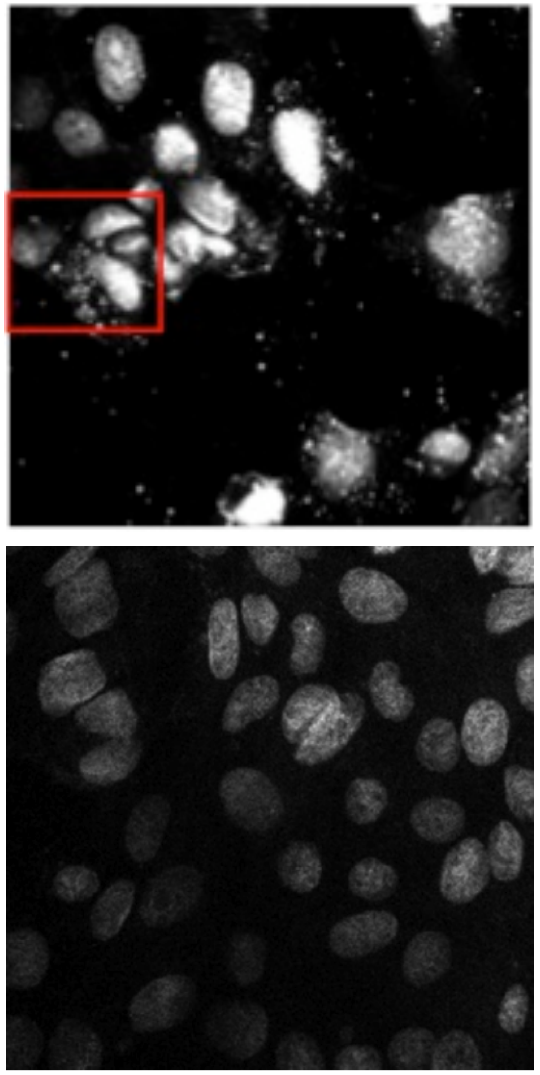




TPL - AuNRs in MDCK Cells

- MDCK cells were treated with AuNRs
- Incubated for 3 hrs
- Washed and fixed
- Stained with DAPI

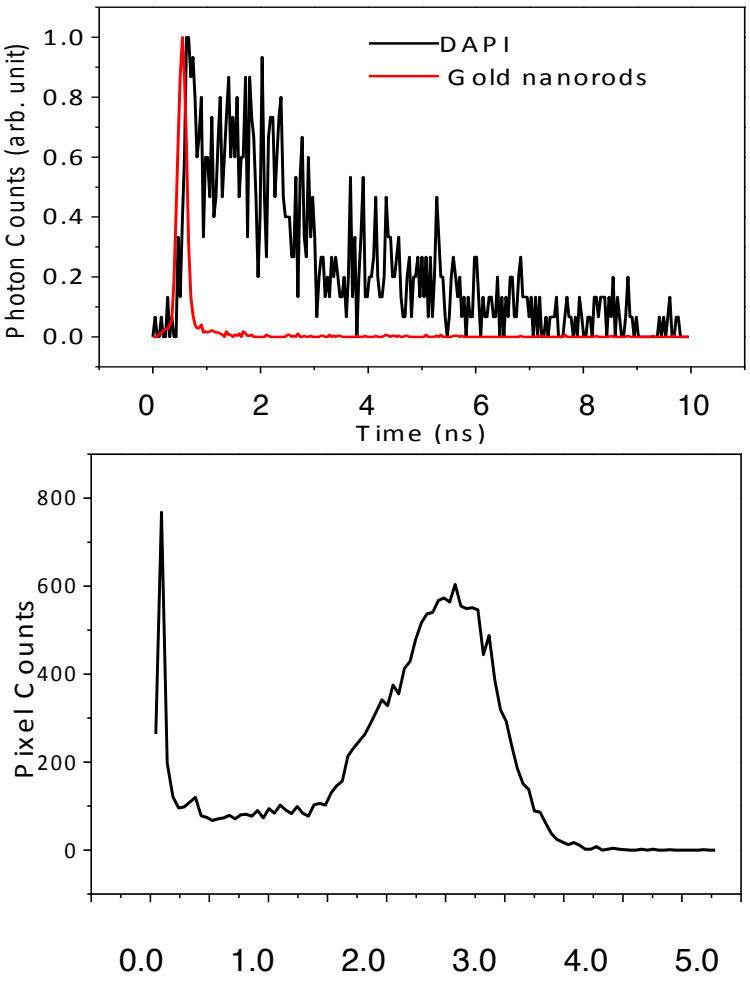


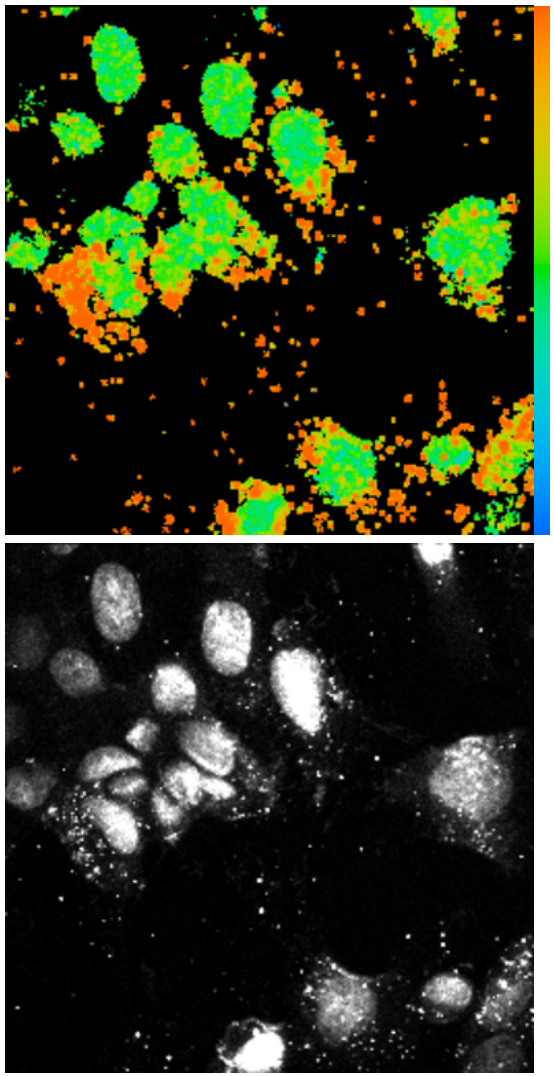


67μm x 67μm

Fluorescence Lifetime Imaging Microscopy (FLIM)

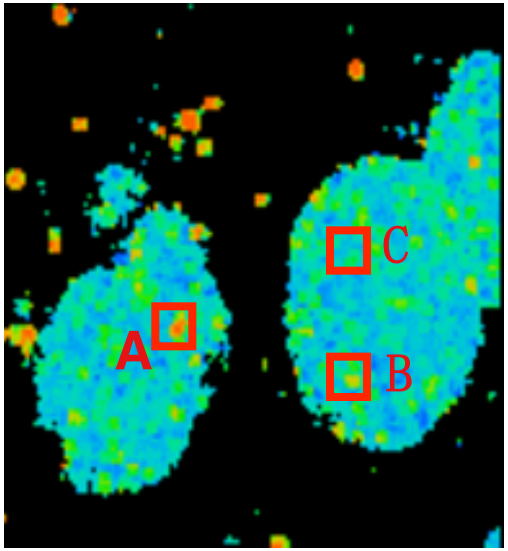
- AuNRs in MDCK cells

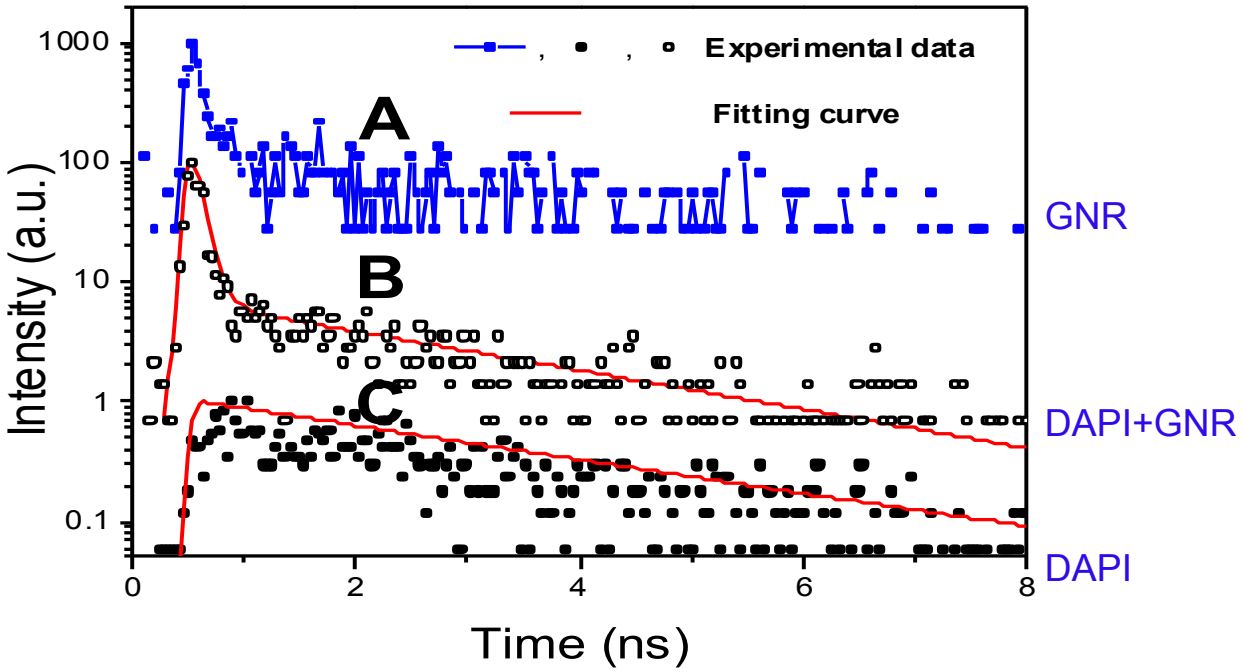




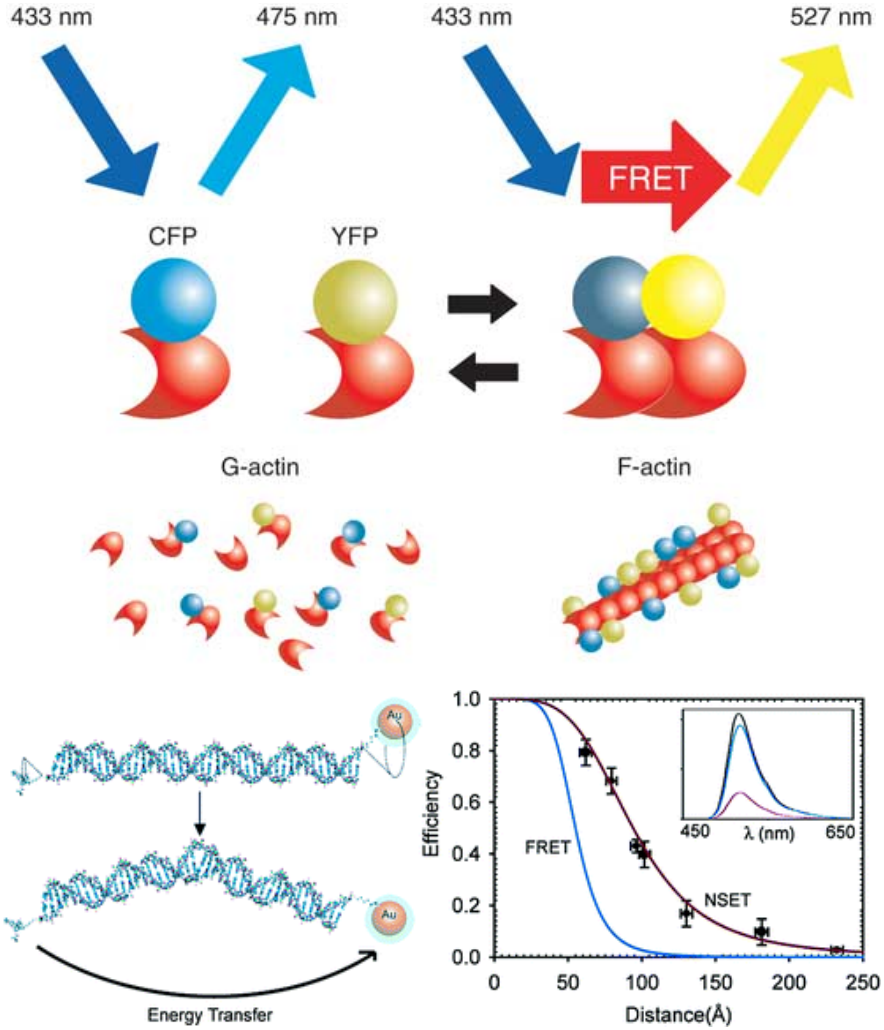
Y. Zhang, J. Yu, D. J. S. Birch and Y. Chen, J. BioMed. Opt. 15, 020504 (2010)

FLIM - AuNR in MDCK cells





Energy Transfer as Molecular Ruler



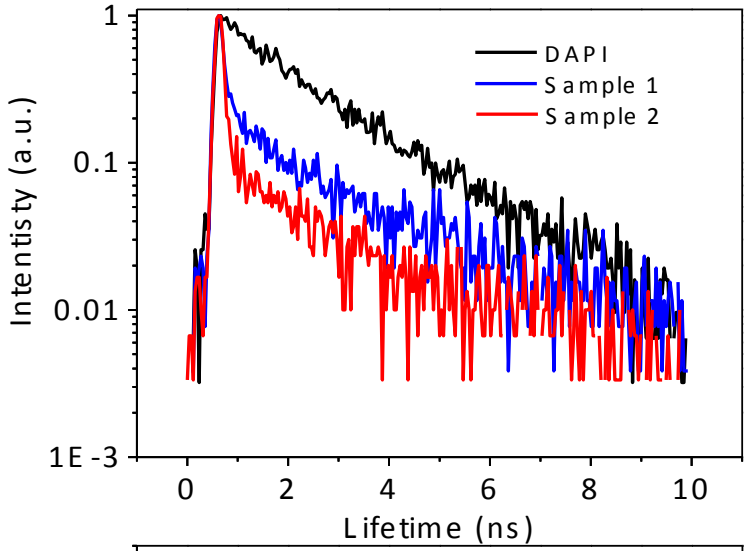
Förster Resonance Energy Transfer (FRET)

$$k_T(r) = \frac{1}{\tau_D}\left(\frac{R_0}{r}\right)^6$$

Surface Energy Transfer (SET)

$$k_T(r) = \frac{1}{\tau_D}\left(\frac{d_0}{d}\right)^4$$

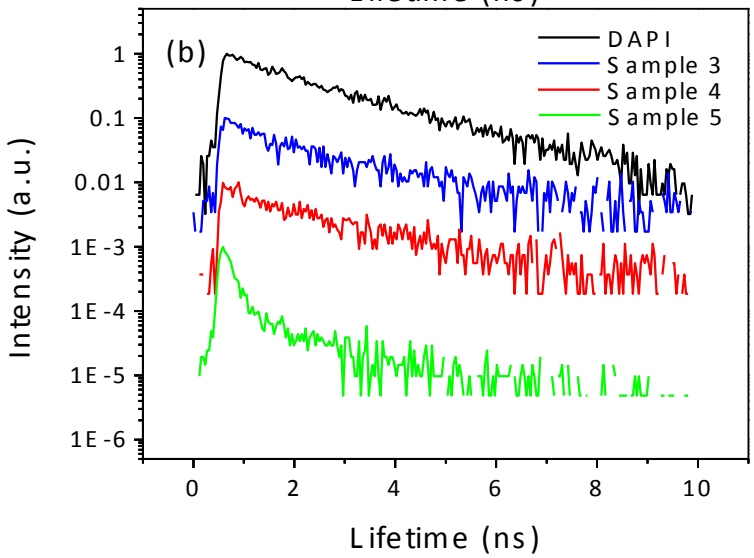
Surface Energy Transfer under Two-Photon Excitation

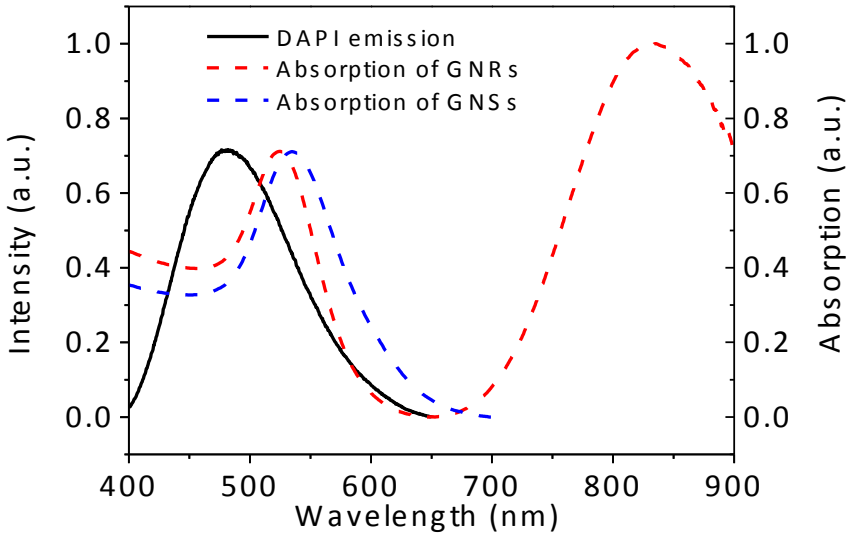


DAPI in solution, 1.7ns

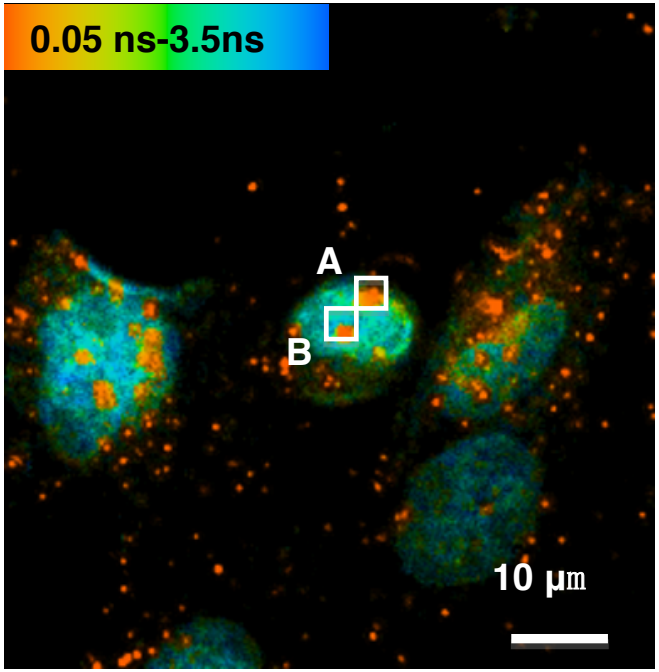
DAPI in S1, 1.55ns (1ml GNR)

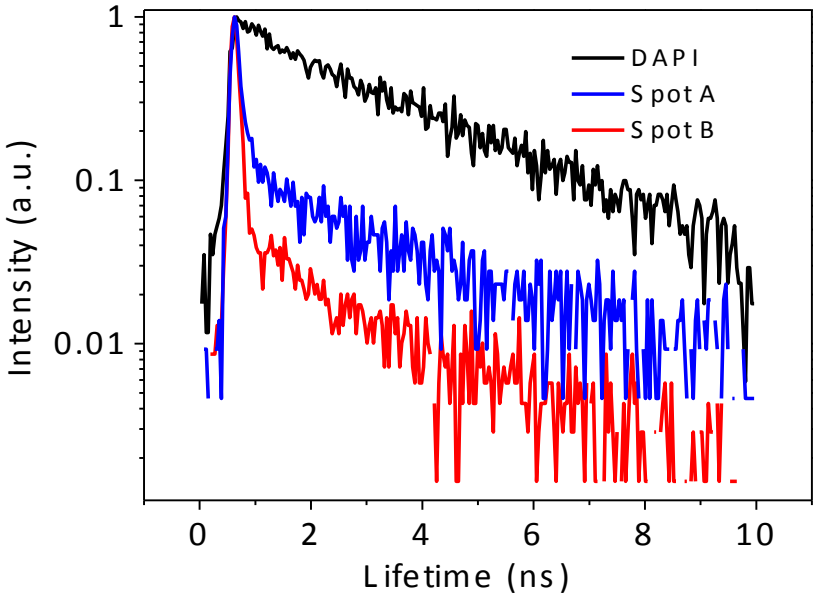
DAPI in S2, 0.9ns (1.4ml GNR)





SET from DAPI to GNR in Cells





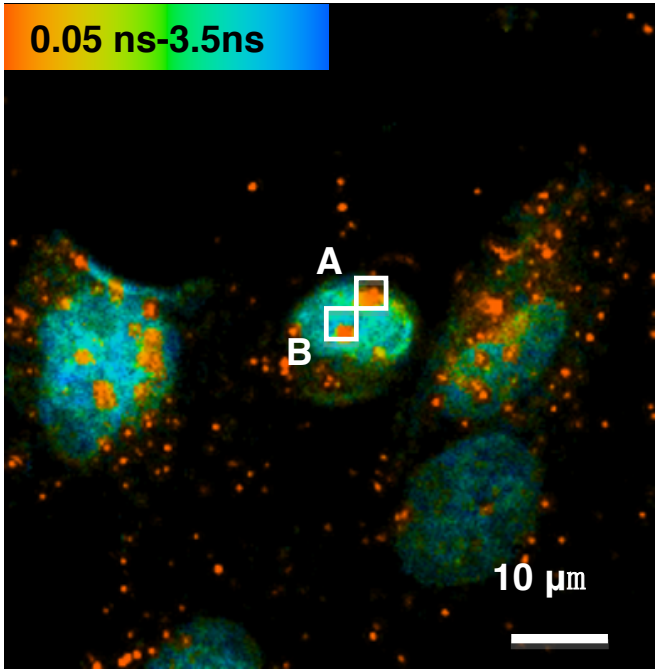
DAPI in cells, 2.5ns

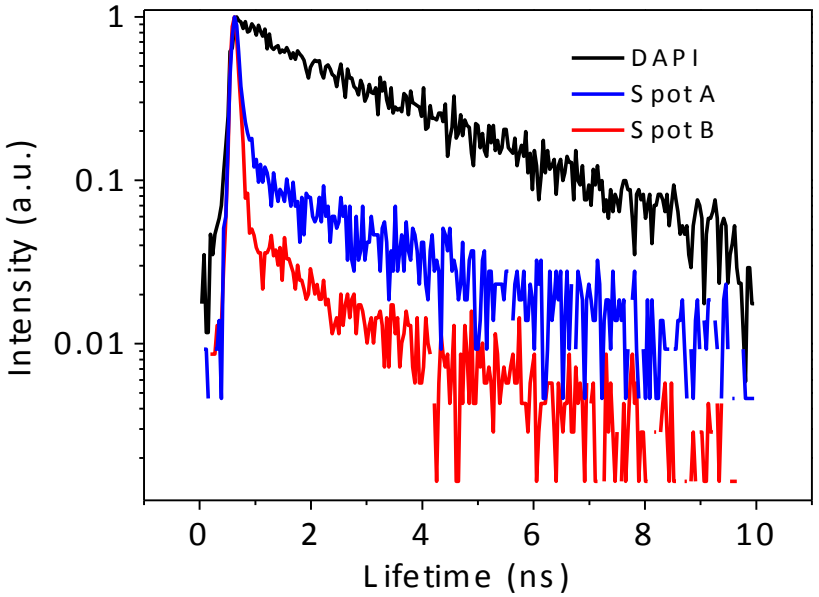
DAPI in A, 2.5ns

DAPI in B, 0.9ns

Y. Zhang, D. J. S. Birch and Y. Chen, Appl. Phys. Lett. 99, 103701 (2011)

SET from DAPI to GNR in Cells





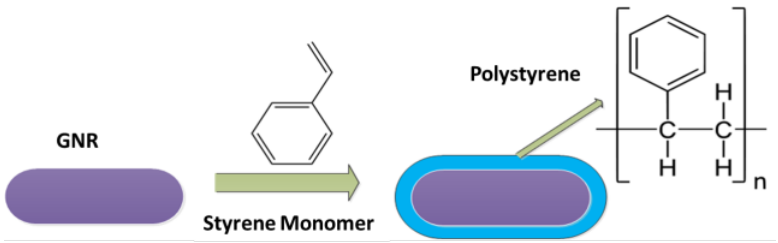
DAPI in cells, 2.5ns

DAPI in A, 2.5ns

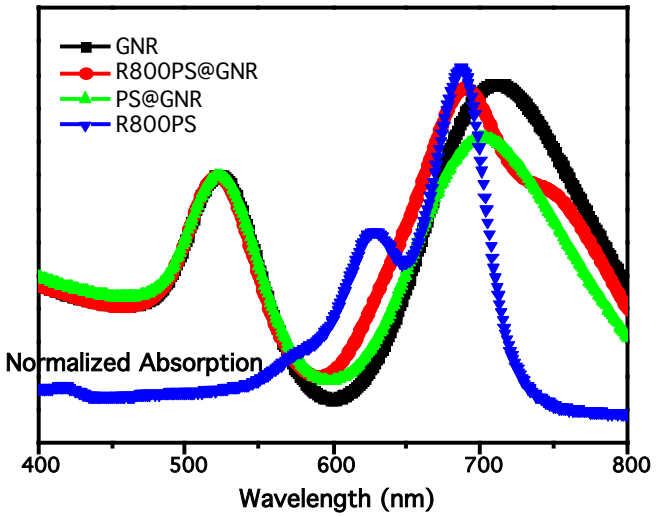
DAPI in B, 0.9ns

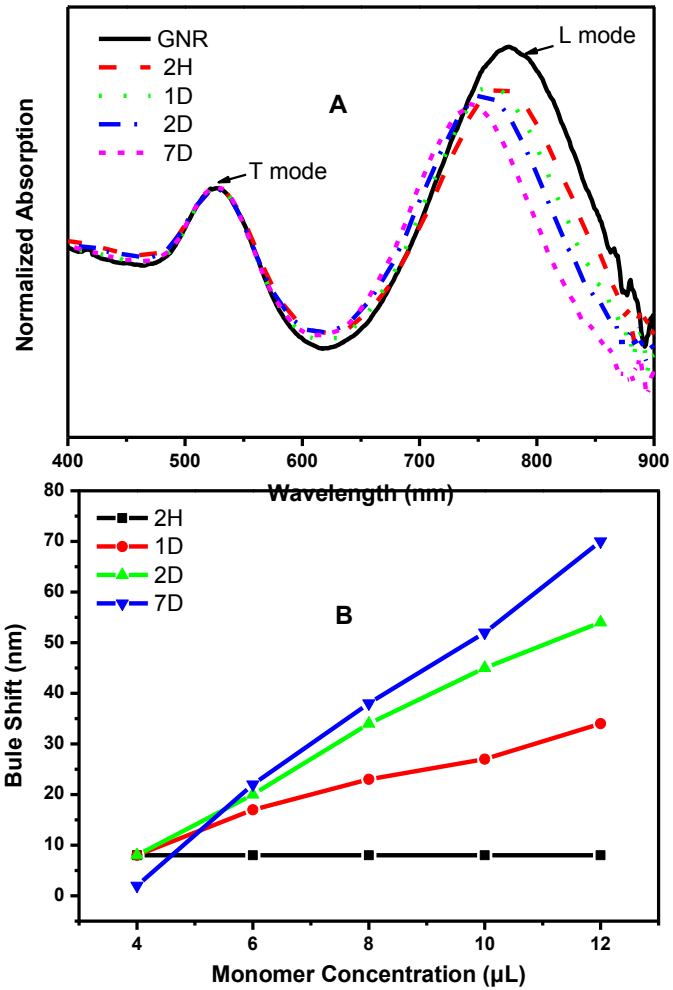
Y. Zhang, D. J. S. Birch and Y. Chen, Appl. Phys. Lett. 99, 103701 (2011)

Dye-dope Polystyrene-coated Gold Nanorods



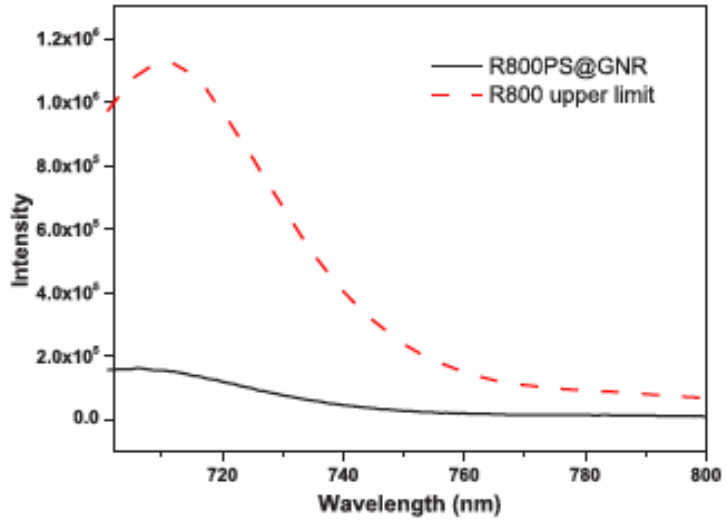
Scheme of polystyrene coating





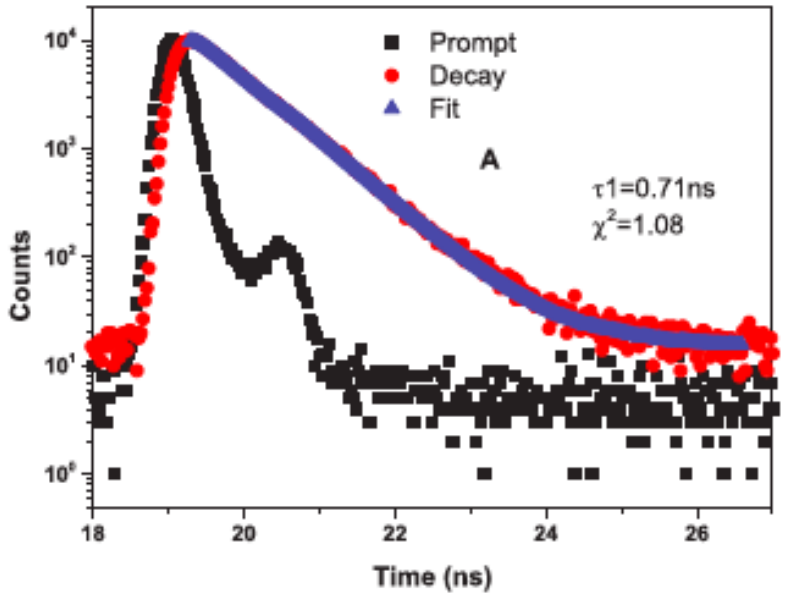
P, Gu, D. J. S. Birch and Y. Chen, Methods Appl. Fluoresc. 2, 024004 (2014)

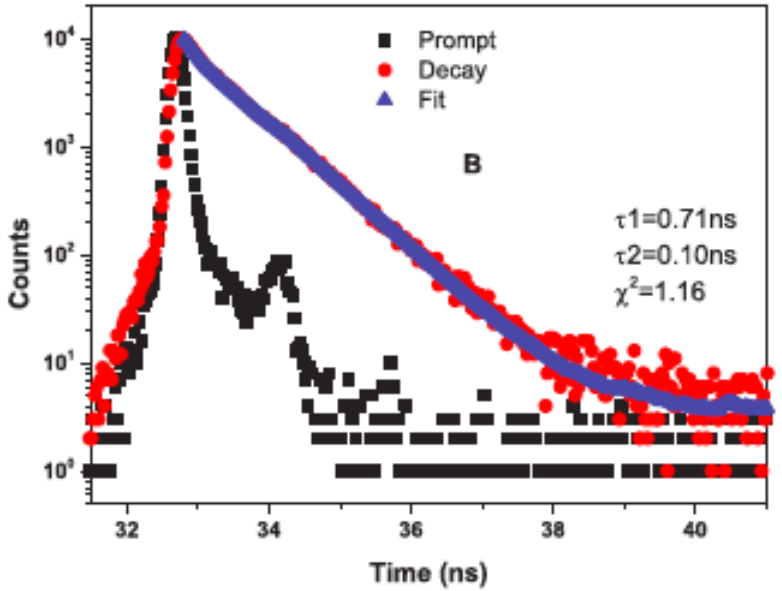
Surface Plasmon Energy Loss Compensation



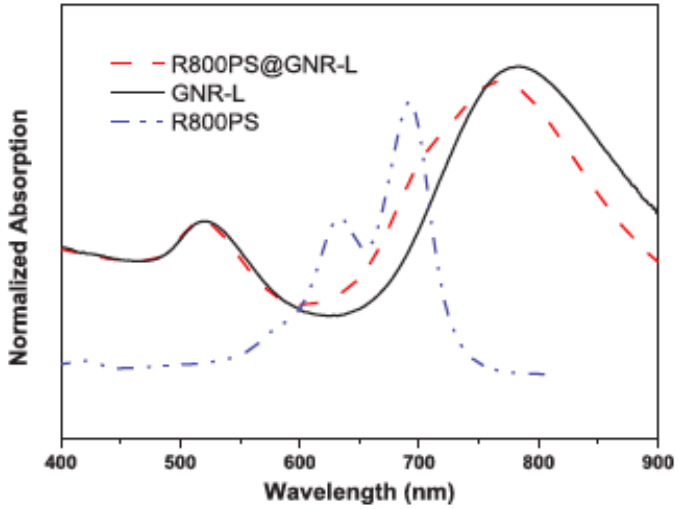
A, 0.71ns

B, 0.71ns (89%); 0.10ns (10.8%)



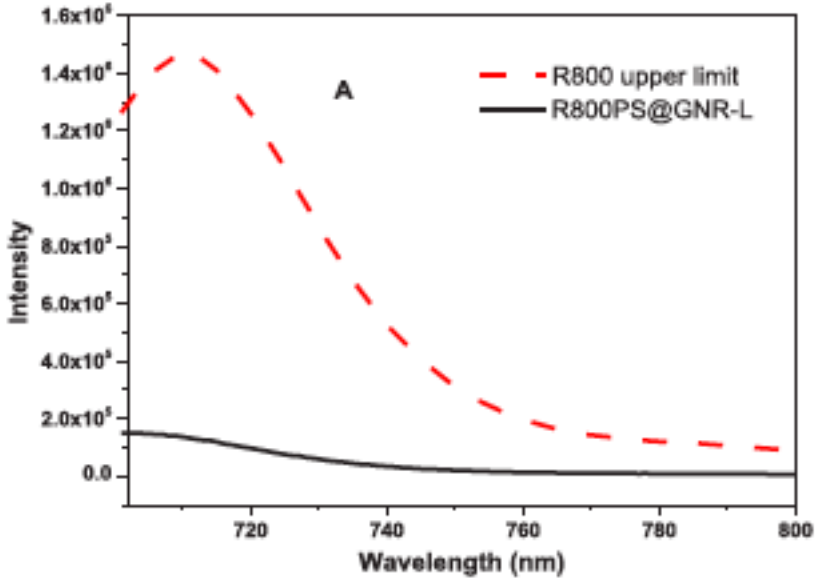


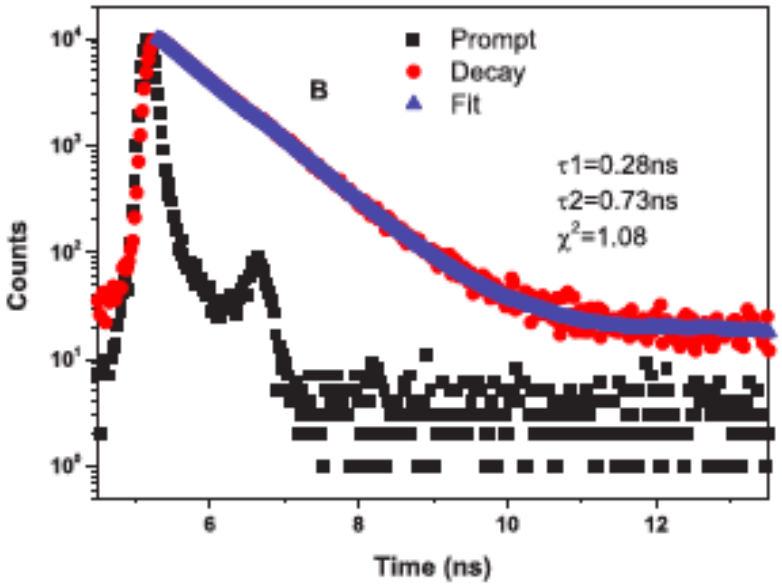
Influence of Surface Plasmon on Energy Transfer



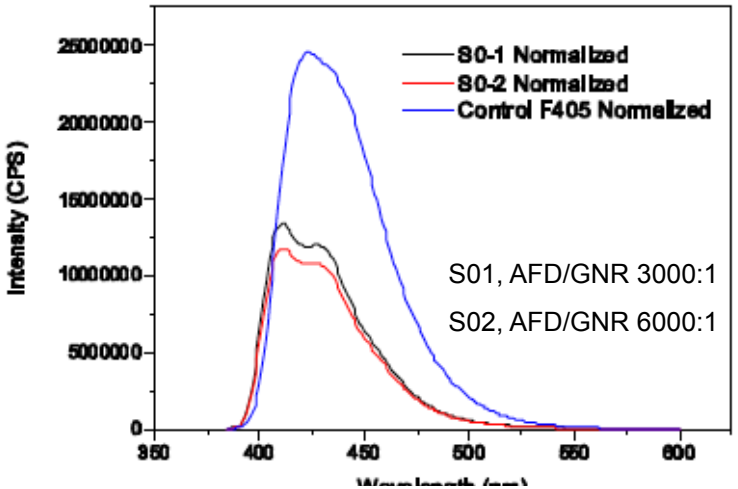
A, 0.71ns

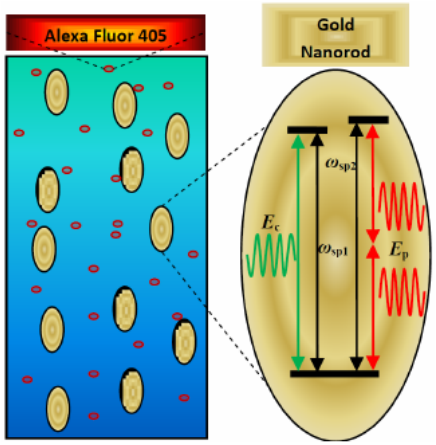
B, 0.73ns (95.8%); 0.28ns (4.2%)

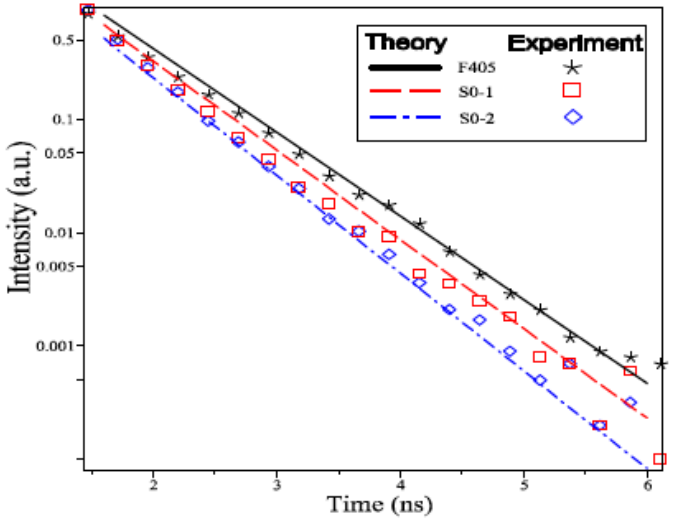


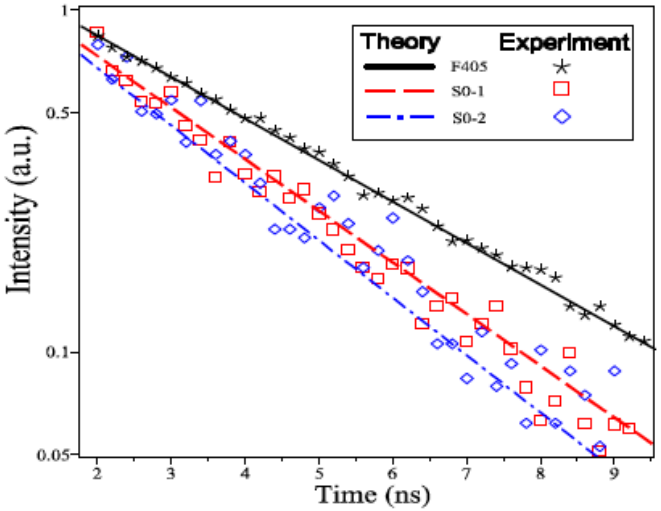


Alex Fluor405 – GNR Hybrid System



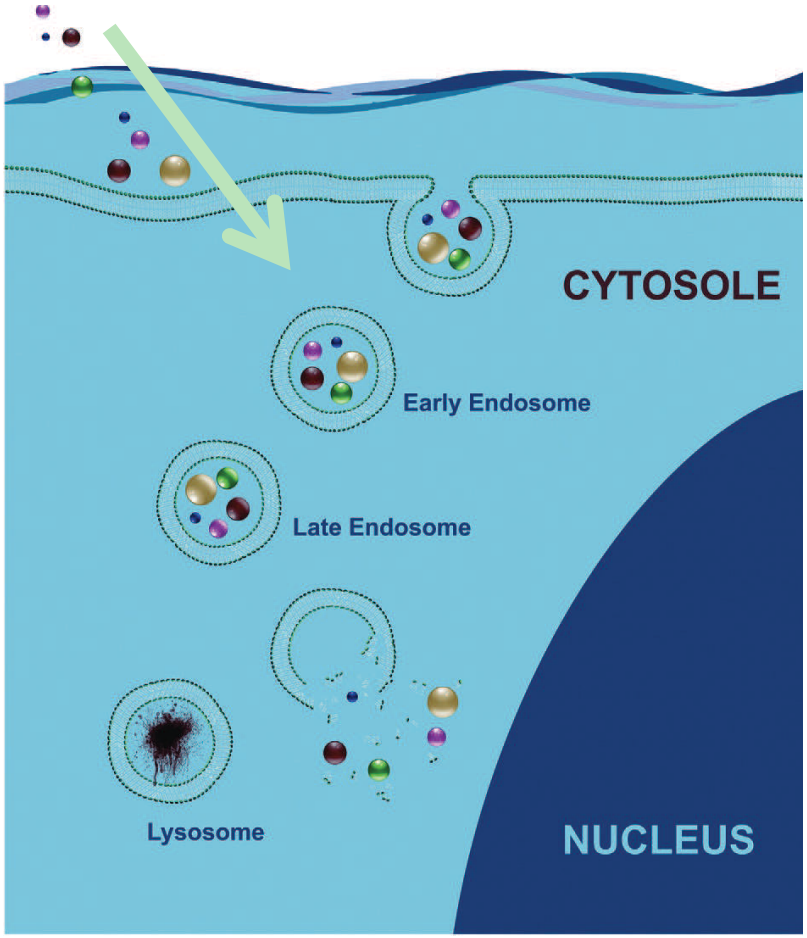


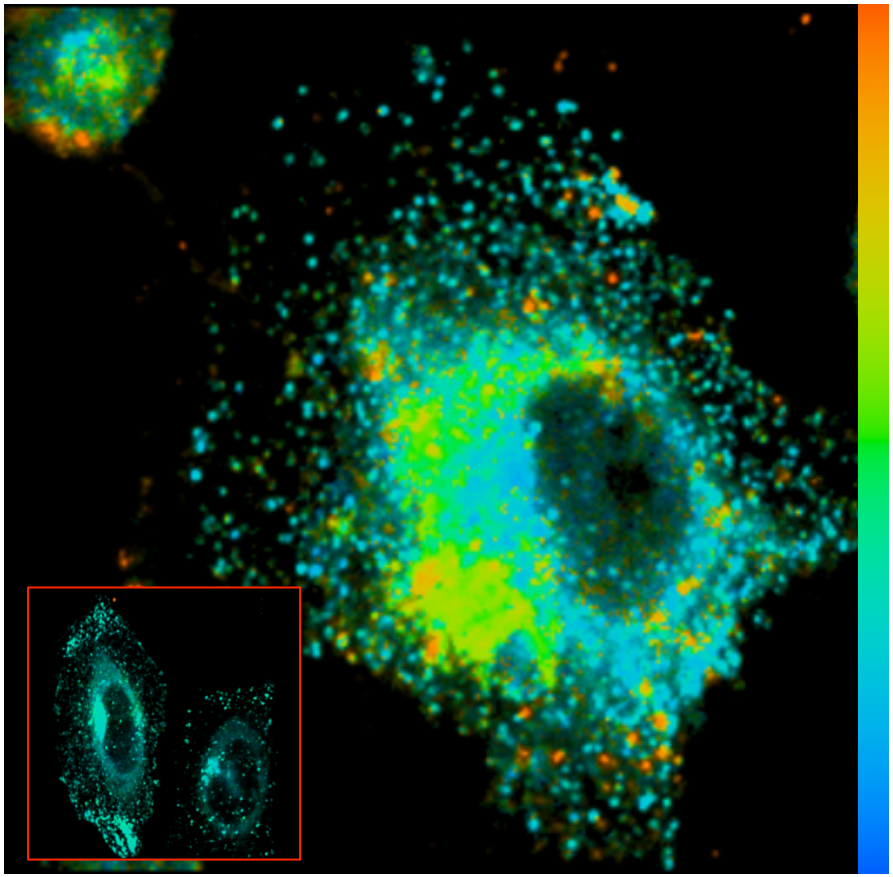




C. Racknor, M. R. Singh, Y. Zhang, D. J. S. Birch and Y. Chen, Methods Appl. Fluoresc. 2, 015002 (2014)

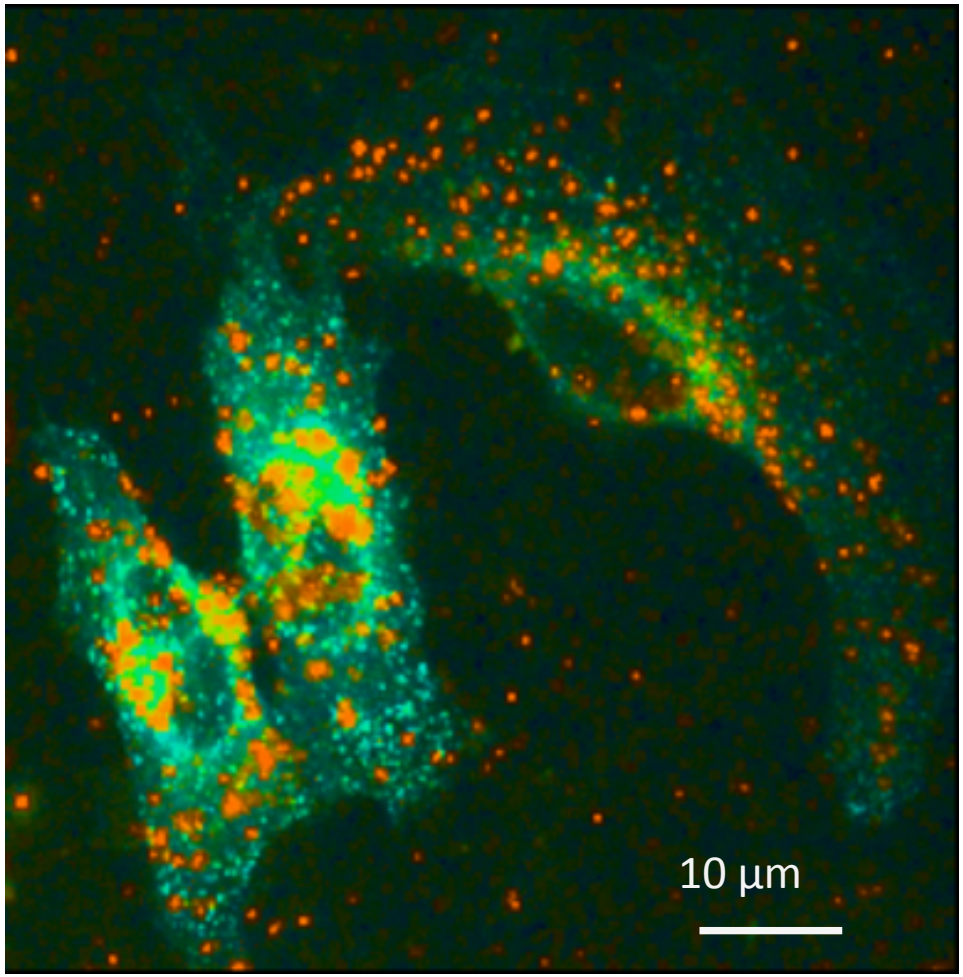
Intra-cellular Pathway





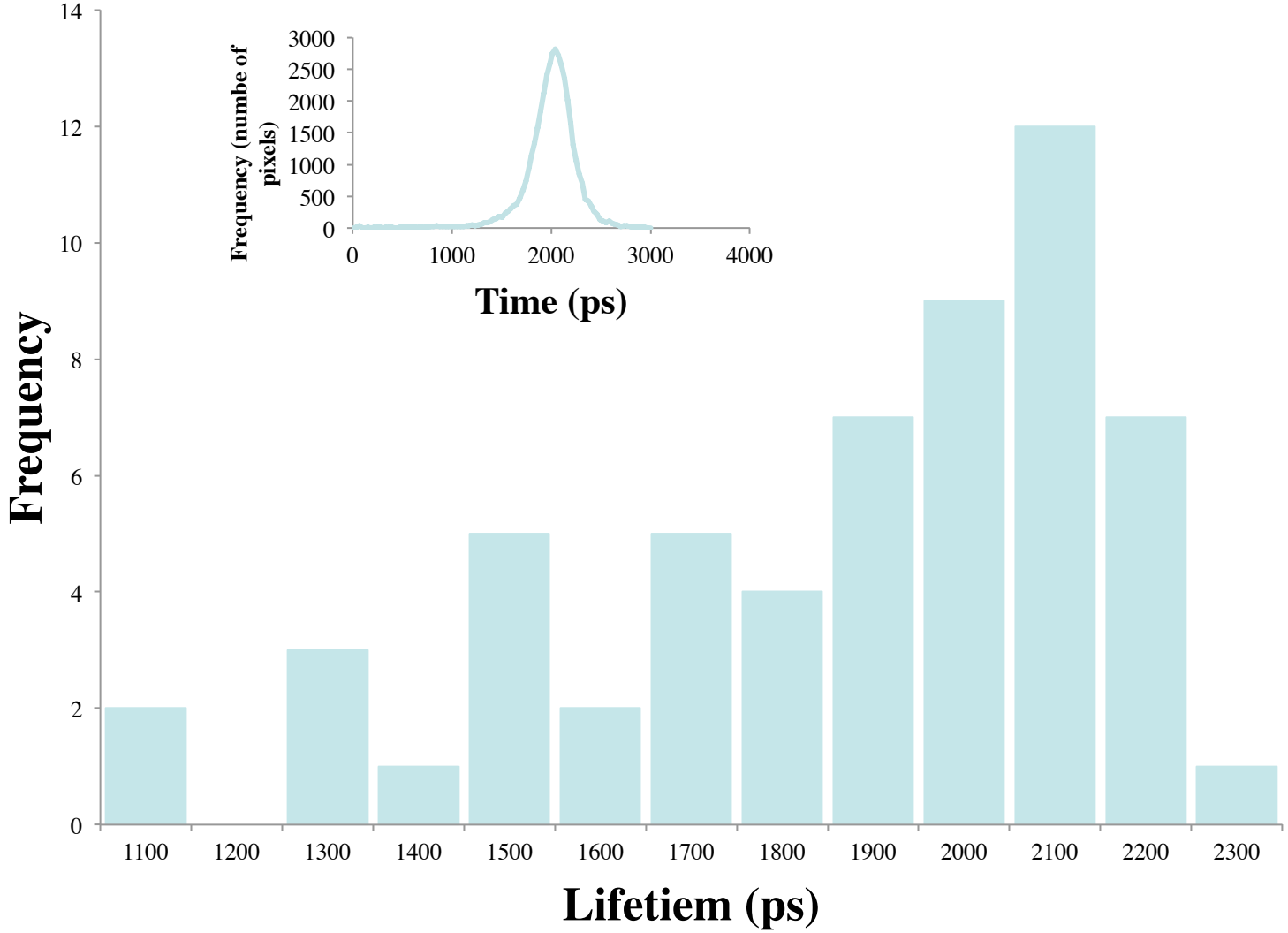
FLIM image of two-photon excited GFP stained HeLa cells, incubated with AuNRs for 60mins.

GNR Cellular Uptake - Endocytosis Pathway



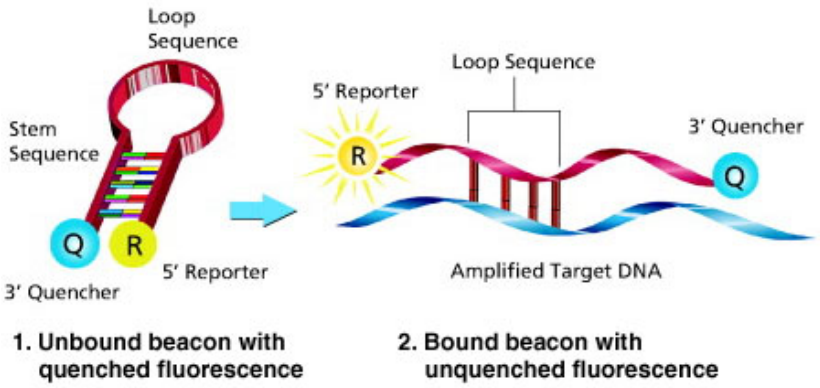
Multilayer coated gold nanords in HeLa cells, incubate time 60min

GNR Cellular Uptake - Endocytosis Pathway



GNR Based RNA Nanoprobes

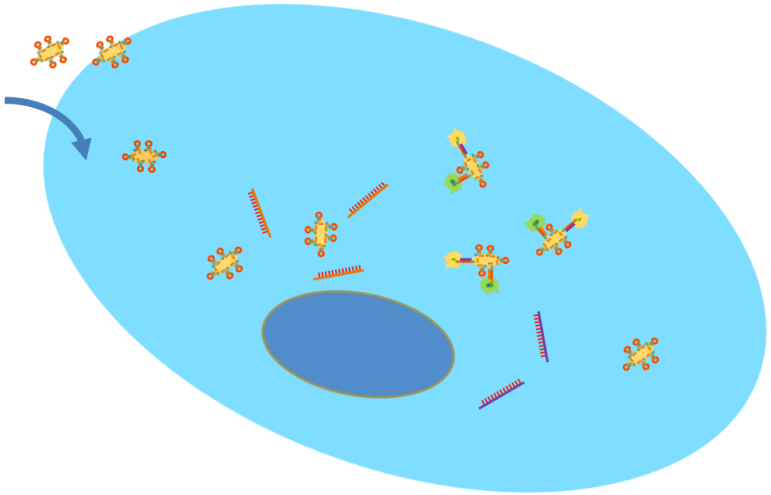
Molecular beacon



Disadvantages:

- The quenching efficiencies of traditional organic quenchers usually vary significantly from one dye to another.
- Require additional agents for cellular internalization.

GRN based RNA nanoprobe



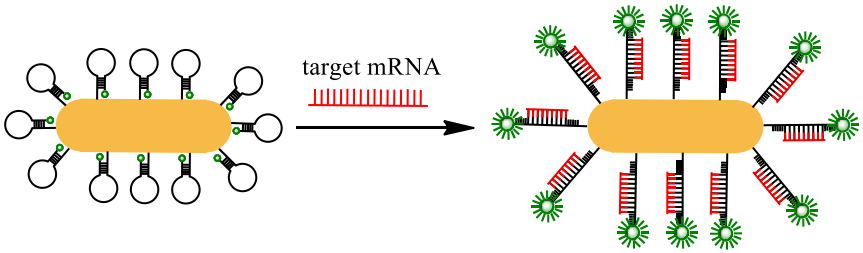
Advantages:

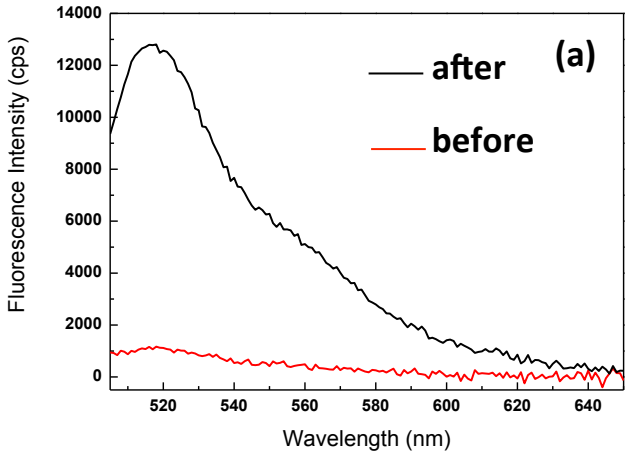
- Strong quenching
- Long interaction range
- Photostable
- No need of transfection agent
- TPL to trace nanoprobe
- Multiple targeting
- Potential for multifunctional platform

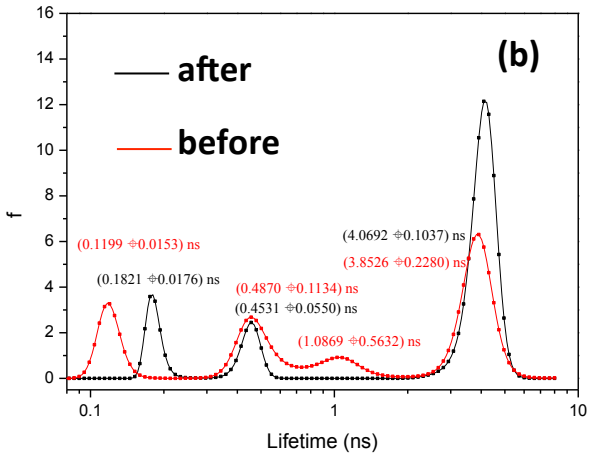
GNR Based RNA Nanoprobes

DNA design,

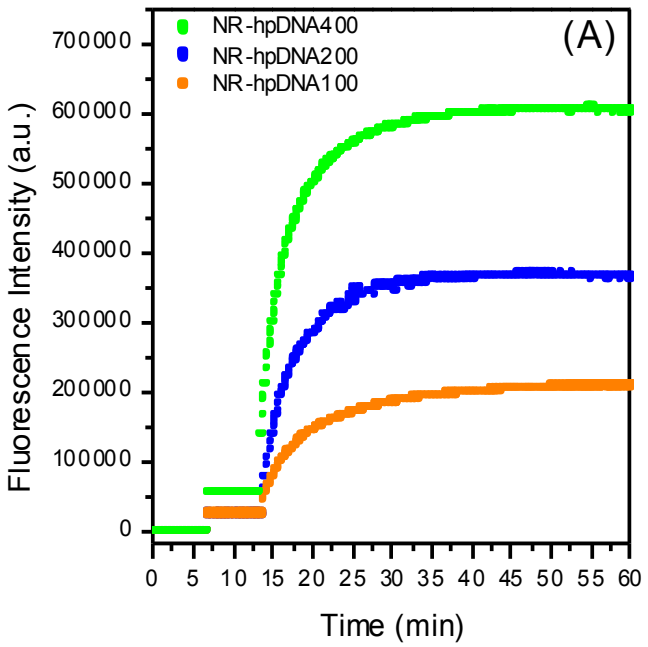
HS-5’TTTTTTTaaagttaacTTGGTGAAGCTAACGTTGAGGgttaacttt 3’ -**Fluorescein**



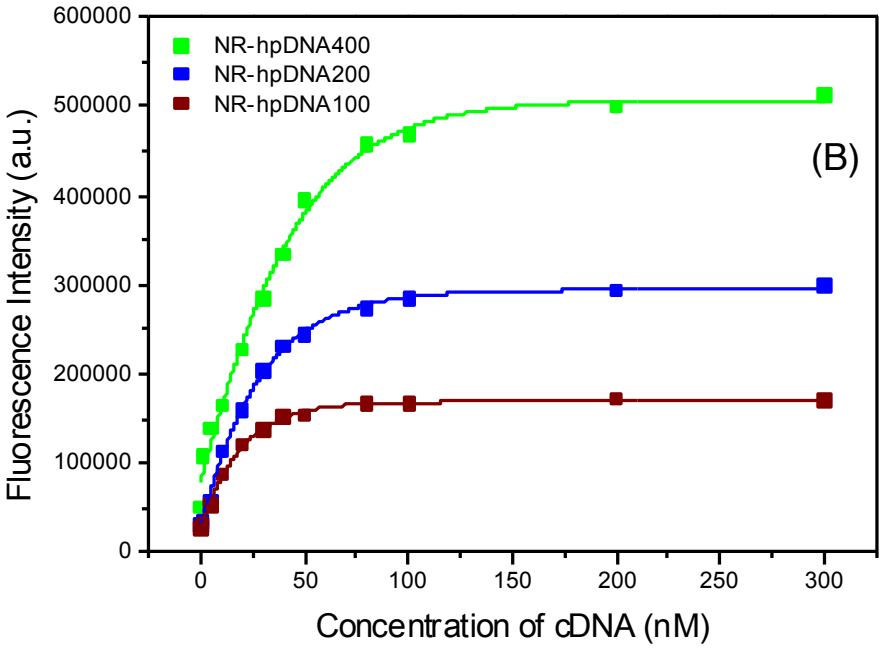




GNR Based RNA Nanoprobes

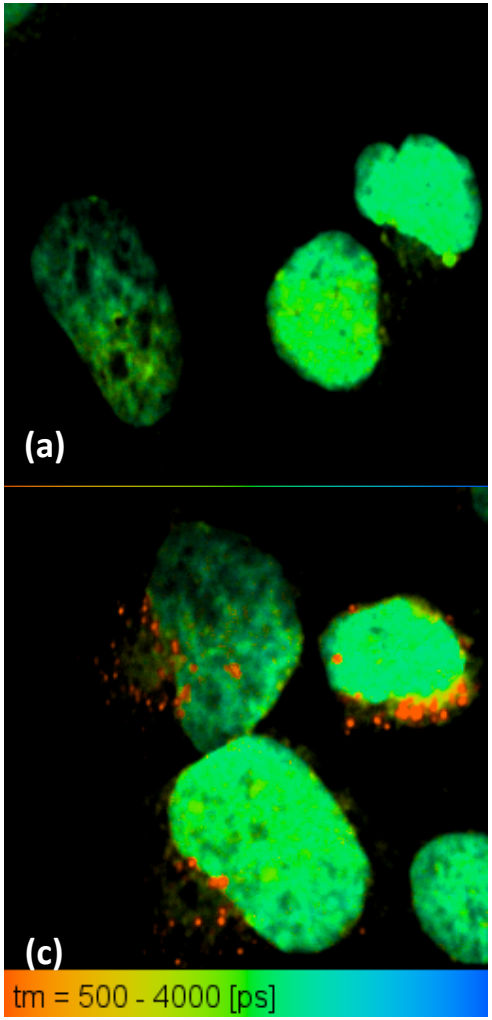


Kinetic measurements of hybridization of GNR-hpDNA (0.22 nM) with cDNA (880 nM)

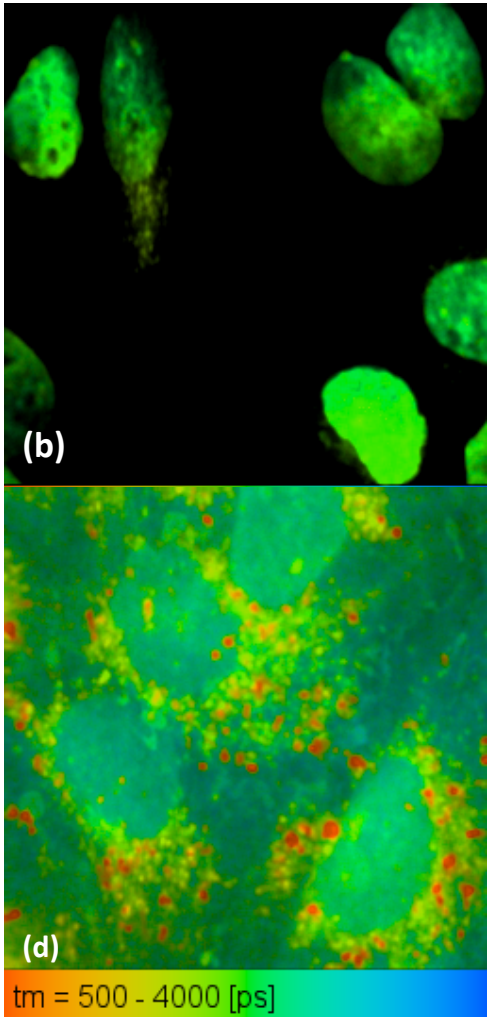


Dose response of the nanoprobes (0.22 nM) with different surface packing densities of hpDNA.

Uptaken of Nanoprobes by Tumor cells

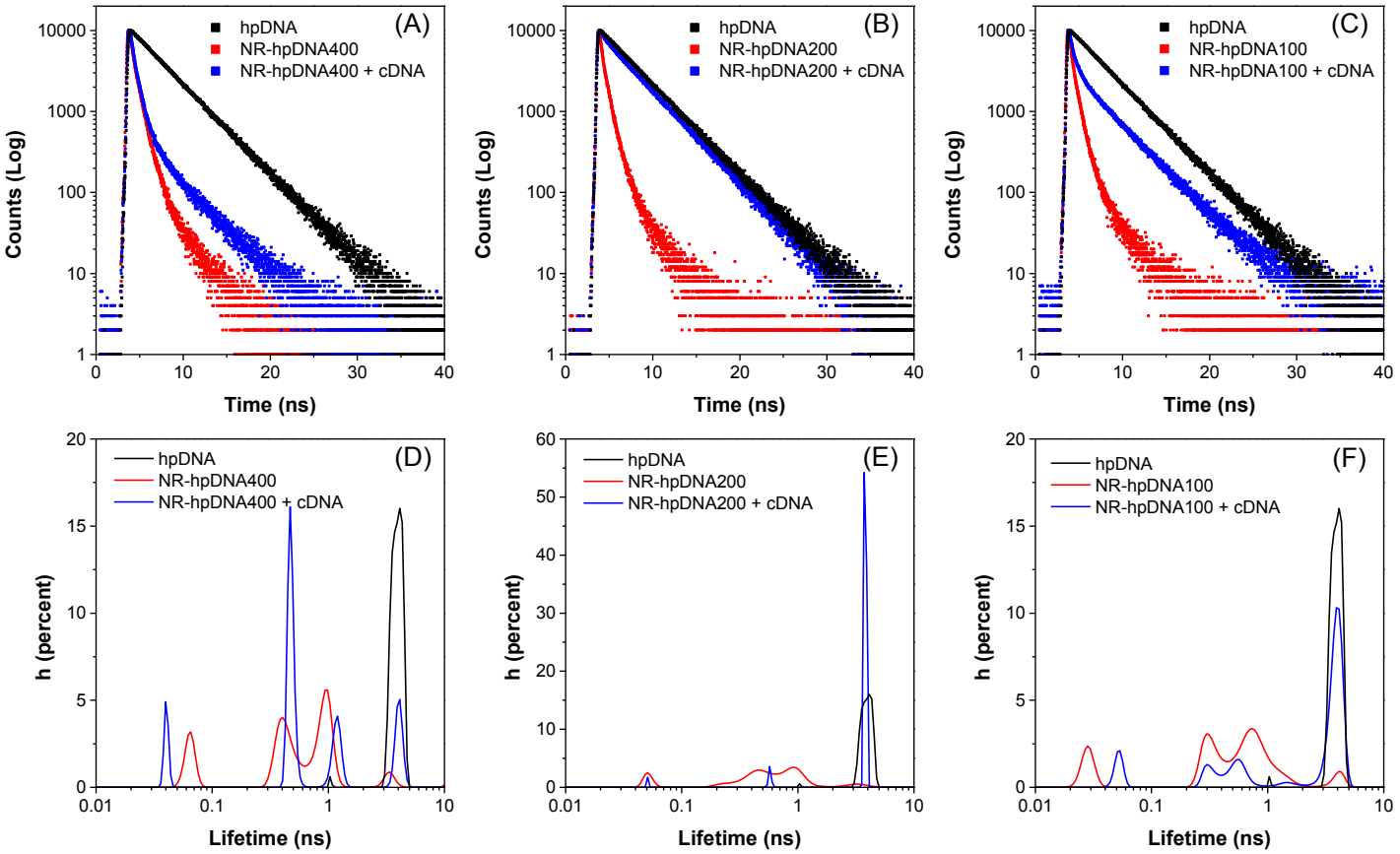


Brain cancer cell



Lung cancer cell

GNR based RNA Nanoprobes



	hpDNA	hpDNA+cDNA	GNR-hpDNA200	GNR-hpDNA200+cDNA
$\bar{\tau}$/ns	3.866	3.929	0.834	3.658

Summary

• We have studied gold nanorods as luminescence label in MDCK cells by FLIM, which provides a better contrast and more detailed features than with that intensity imaging.

• The characteristic short lifetime together with polarization of TPL from gold nanorods can be a promising imaging contrast agent for use in luminescence microscopy in biology.

• Two-photon excited surface plasmon enhanced energy transfer between DAPI and AuNRs is observed in both solution phase and cell culture.

• With comparable size and concentration, gold nanorods are shown to provide more efficient energy transfer than gold nanospheres. We attribute this transfer enhancement effect to the longitudinal surface plasmon mode of GNRs overlapping with the excitation wavelength.

• The energy transfer provides more detailed information in biological studies using GNRs as fluorescence probes, especially when combined with the advantages of two-photon excitation microscopy and more intense TPL from GNRs, as demonstrated here in the study of intra-cellular trafficking of GNRs in HeLa cells via GFP labelled early endosome and mRNA sensing at single cell level.

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